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ROLE OF EDTA IN LEAD MOBILIZATION AND ITS UPTAKE BY MAIZE GROWN ON AN ARTIFICIAL Pb-POLLUTED SOIL

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Abstract

Soil pollution with heavy metals is a serious issue worldwide. Metal pollution has serious implications for the human health and environment. Phytoremediation is considered an economical and environmentally friendly method of exploiting plants to extract contaminants from soil. The purpose of this paper is to study the maize seedling, growing and behaviour in a soil polluted with heavy metals. Maize is known from literature as lead accumulators in artificially polluted soil with 1000, 2000 and 3000 mg/kg Pb of soil and in the presence of different treatments with EDTA as the mobilization agent. This means that the treatment for phytoextraction (Pb concentration, EDTA concentration) is expressed in the biomass. From the statistical calculation it results that in the variant with 1000 mg Pb/kg soil + ratio EDTA/Pb = 0.5 have no significant decrease in leaf weight. In conclusion, EDTA application does not influence hyperaccumulation. The toxicity of 3000 mg Pb/kg is too high and the plant does not tolerate this toxicity. Another ligand/lead ratio has to be chosen and other solutions are sought to stimulate plant growth and increase the accumulation of metals in the plant.

Key words*: soil, pollution, lead, maize.*

INTRODUCTION

Soil pollution with heavy-metal is one of the main global environmental problems (Wan et al., 2016).
Regarding their role in biological systems, heavy metals are classified as essential and non-essential. Essential heavy metals are those, which are needed by living organisms in low quantities for vital physiological and biochemical functions. Examples of essential heavy metals are Fe, Mn, Cu, Zn, and Ni (Cempel & Nikel, 2006; Gohre & Paszkowski, 2006). Non-essential heavy metals are those, which are not needed by living organisms for any physiological and biochemical functions. Examples of nonessential heavy metals are Cd, Pb, As, Hg and Cr (Sanchez-Chardi et al., 2009; Dabonne et al., 2010).
Heavy metal concentrations beyond threshold limits have adverse health effects because they interfere with the normal functioning of living systems.
Phytoextraction of heavy metals can be practiced in two ways, natural and induced.
In natural or continuous phytoextraction, plants are used for removal of heavy metals under natural conditions, no soil amendment is made.
In induced or chelate assisted phytoextraction, different chelating agents such as EDTA, citric acid, elemental sulfur, and ammonium sulfate are added to soil to increase the bioavailability of heavy metals in soil for uptake by plants (Sun et al., 2011).
Phytoremediation of heavy metals in soils can be categorized into four major routes: uptake of heavy metal, bioaccumulation of heavy metal, *in situ* inactivation or immobilization of heavy metal, minimizing the bioavailability and external transport of heavy metal, and transformation of volatile forms and discharge into the atmosphere (Shah & Daverey, 2020).
Figure 1 presents the traditional concept that have limitations approaches of phytoremediation and to minimize these limitations and to ensure large scale application of phytoremediation, a lot of research was conducted in this field and result in recent advancements in phytoremediation as a modified concept (Sarwar et al., 2017).

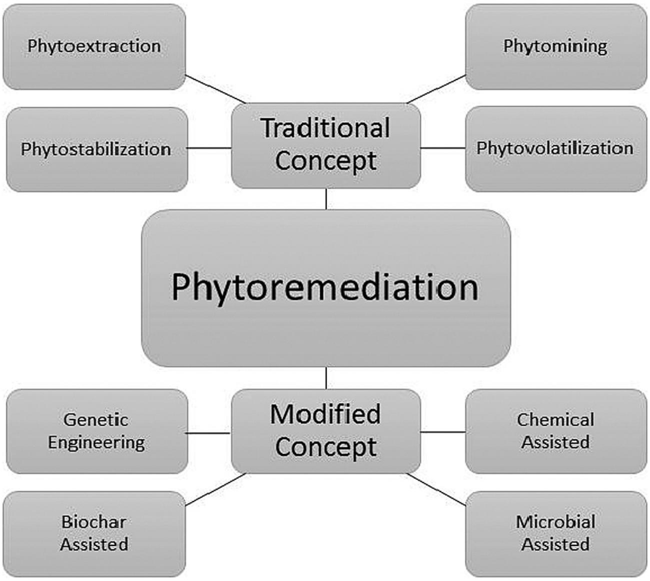

Figure 1. Approaches for heavy metals phytoremediation (Sarwar et al., 2017)

In Figure 2 is illustrated diagram showing the relationship between immobilization, bioavailability and phytoremediation of toxic heavy metals. Both phytoextraction and phytostabilization processes are part of phytoremediation technique employed to manage contaminated soils. The sources of the common heavy metals input to soils, their interactions and bioavailability in soils, and the remediation of metals contaminated soils through manipulating their bioavailability using a range of mobilizing or immobilizing soil amendments is a research challenge (Bolan et al., 2014).

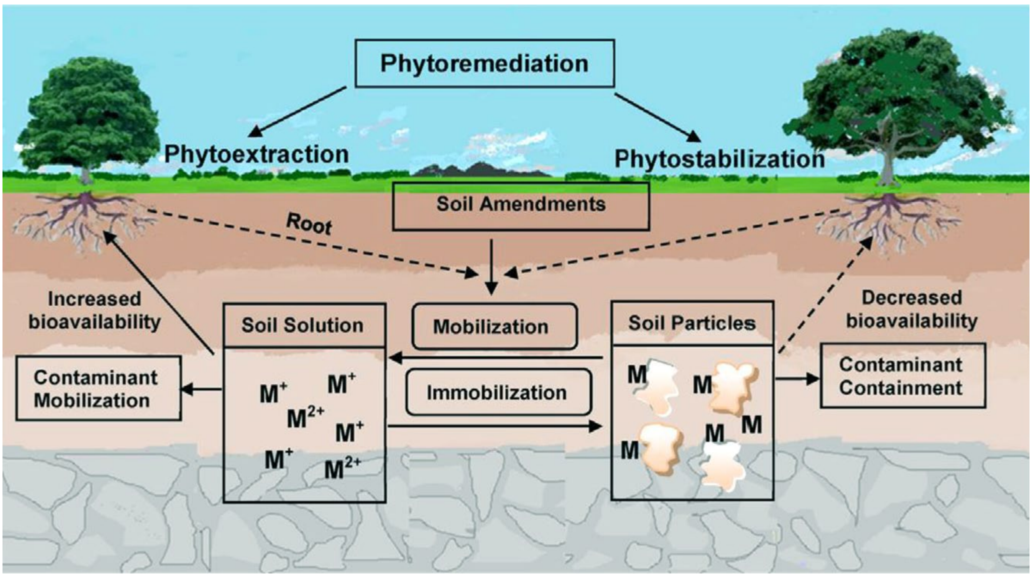

Figure 2. Illustrated diagram showing the relationship between immobilization, bioavailability and phytoremediation of toxic heavy metals (Bolan et al., 2014)

MATERIALS AND METHODS

The purpose of this paper is to study maize seedling, growing and behavior. Maize is known in the literature as being accumulator for lead in the soil polluted artificially with 1000, 2000 and 3000 mg Pb/kg of soil and in the presence of different amounts of EDTA as a mobilization agent. Experiment consists in 8 variants in three repetitions. The test plant chosen is maize. Different variants for maize: V17-V32 variants: 1000-3000 mg Pb/kg soil + EDTA (in different ratios to Pb). The experiment scheme are as follows in Table 1.

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Table 1. Experimental scheme

Experimental variant	Lead concentration (mg/kg)	EDTA (Ligand/Lead ratio)
V17	0	0
V18	1000	0
V19		0.5
V20		1
V21		2
V22		10
V23	2000	0
V24		0.5
V25		1
V26		2
V27		10
V28	3000	0
V29		0.5
V30		1
V31		2
V32		10

The soil used in the experiment is a cambic chernozem from Fundulea area, Călărași County. In Table 2 are presented the physical characteristics of soil.

In table 3 are presented chemical characteristics of cambic chernozem from Fundulea.

Table 2. Physical characteristics of cambic chernozem from Fundulea area, Călărași county (n = 3)

	Particle - size distribution (in mm) (% of the mineral part of the soil)				Symbol – subclass texture	Carbonates (%)
	Coarse sand	Sand	Silt	Clay		
	2.0-0.2 mm	0.2-0.02 mm	0.02	< 0.002 mm		
Mean	0.3	33.1	30.7	35.9	LL - Medium Clay (Romanian Soil Taxonomy System, 2003)	-

Table 3. Chemical characteristics of cambic chernozem from Fundulea area, Călărași county (n = 3)

Characteristics	M.U.	Mean value
pH_{H2O}	-	6.84
Total nitrogen content	%	0.255
Organic carbon content	%	3.98
Mobile phosphorous content	mg kg^{-1}	17
Mobile potassium content	mg kg^{-1}	140

In Table 4 are presented the contents of heavy metals in soil that will be used in the experiment. All the values registered for cadmium, copper, cobalt, nickel, lead, manganese and zinc are beyond the alert threshold.

Table 4. The content of heavy metals in cambic chernozem from Fundulea area (n = 3)

Heavy metals content	M.U.	Mean value
Cadmium	mg kg^{-1}	0.3
Copper	mg kg^{-1}	27
Cobalt	mg kg^{-1}	10
Nickel	mg kg^{-1}	34
Lead	mg kg^{-1}	25
Manganese	mg kg^{-1}	761
Zinc	mg kg^{-1}	83

RESULTS AND DISCUSSIONS

The maize vegetation period was 8 weeks.
The evolution of the plants from sowing, emergence to harvesting was followed. Regarding leaf appearance and emergence, there was a strong influence of Pb treatment with EDTA.
After harvesting maize plants measurements of plant height and weight of the resulting biological material as well as lead dosages were made to determine the amount of lead accumulated in plants (Figures 3-5).
Following the variance analysis (Tukey test, Fisher test), statistical data showed a different evolution of these parameters depending on the treatment applied. There are statistically significant differences both in the weight of the biological material at harvest and at plant height, but also in the lead content of the plants.

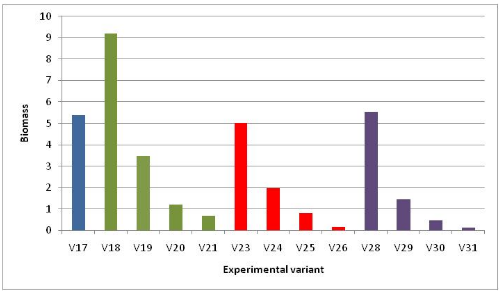

Figure 3. Biomass evolution of maize plants on a soil polluted with 1000, 2000 and 3000 mg Pb/kg and EDTA/Pb ratios by 0, 0.5, 1 and 2

For soil polluted with 1000 mg/kg Pb and treated with increasing amounts of EDTA (ligand) such that the EDTA / Pb ratio reaches 0; 0.5; 1 and 2, leaf weight decreases significantly from the control of each variant in which the ligand (EDTA) was applied in the 0.5, 1 and 2 ratios (Fisher test). At V18 (without EDTA), the increase in leaf weight against the control (V17) is due to the higher nitrogen content of the Pb nitrate that acted as a fertilizer in this case. The significant decrease in leaf weight compared to the V17 control appears only in variants V20 and V21, where the EDTA/Pb ratio is higher: 1 and 2. The decrease is not significant compared to the V17 variant in variant V19: Sol (+ 1000 mg/kg Pb) + EDTA/Pb = 0.5.
There is a distinctly significant decrease in leaf weight with increasing EDTA concentration (increase in EDTA/Pb ratio). At the same time, in these variants, including the V24 EDTA/Pb = 0.5 variant, the biomass decrease is distinctly significant which means that the use of maize as a hyperaccumulative plant on a 2000 mg Pb/kg soil loaded can be tested in EDTA at an EDTA/Pb ratio lower than 0.5, in a variant where the decrease in biomass is not significant. Figure 3 shows the soil treated with a lead content (2000 mg/kg) and different EDTA contents (EDTA/Pb = 0, EDTA/Pb = 0.5, EDTA/Pb = 1, EDTA/Pb = 2 molar ratio).

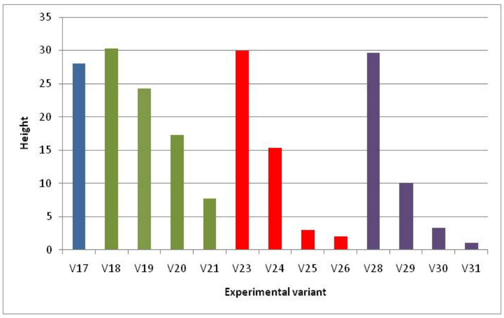

Figure 4. Height of maize plants on a soil polluted with 1000, 2000 and 3000 mg Pb/kg and EDTA/Pb ratios by 0, 0.5, 1 and 2

Plant height decreases significantly from the control. The height evolution is similar to the weight of the leaves. If the treated soil with a lead content (2000 mg/kg) and different contents of EDTA (EDTA/Pb = 0, EDTA/Pb = 0.5, EDTA/Pb = 1; EDTA/Pb = 2 molar ratio), there are differences in the thickness of the leaves. The maize leaf height values also significantly decrease.

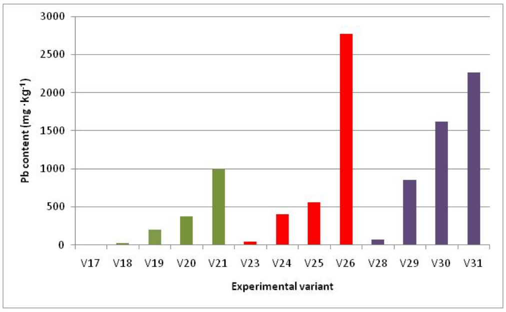

Figure 5. Pb content of maize plants on a soil polluted with 1000, 2000 and 3000 mg Pb/kg and EDTA/Pb ratios by 0, 0.5, 1 and 2

Concerning leaf lead content, there is a distinctly significant increase in each variant compared to the V17 control. Significant increase of Pb relative to the control occurs in variants V19, V20 and V21, where EDTA treatment was performed. The bioavailability of lead increased with increasing EDTA concentration in the soil.

Corroborated, the evolution of the Pb content of leaves with the evolution of biomass weight and plant height, from the test experience, that can be said that only until the EDTA / Pb ratio of 0.5 does not show a significant decrease in biomass, the ligand does not react to this level of negative concentration, although the lead concentration increases.

The content of Pb increases significantly in all leaves, which means that even at concentrations of 2000 mg/kg Pb in soil the bioavailability of lead has increased with the increase in EDTA content. Values higher than 450 mg/kg occur in variants V25 and V26, where the ligand treatment was EDTA/Pb ratio = 1 and EDTA/ Pb ratio = 2.

The lead concentration significantly increases in all variants compared to the control. Values higher than 1053 appear only between V30, EDTA/Pb = 1; V31: EDTA/Pb = 2 and the V17 control on the one hand and between the same variants (V30, V31) and V28 where the soil with the concentration of 3000 mg Pb/kg does not contain EDTA.

Since the decrease of biomass is significant even from V29: EDTA/Pb = 0.5 and the lead concentration increases significantly (> 1053) from V30: EDTA/Pb = 1 the conclusion is that the application of EDTA can not influence the hyperaccumulation; the toxicity of 3000 mg Pb/kg is too high and the plant does not tolerate this toxicity. Thus, another ligand / lead ratio has to be chosen and other solutions are sought to stimulate plant growth and increase the accumulation of metals in the plant.

On the treated soil with different lead concentrations (1000 mg/kg Pb, 2000 mg/kg Pb, 3000 mg/kg Pb) and the same EDTA content (EDTA/Pb = 0.5 molar ratio), the Pb concentration in the leaves increases with soil Pb. Significant increases are at all variants versus control. This means that the choice of the best treatment for phytoextraction (Pb concentration, EDTA concentration) is the amount of biomass. From the statistical calculation it results that in the experimental variant of 1000 mg/kg Pb soil + EDTA/Pb ratio = 0.5 no significant decrease in leaf weight occurs.

In the case of increasing the lead concentration at the same EDTA/Pb ratio of 0.5, the Fisher Test shows a significant decrease in biomass weight and a distinctly significant height of maize plants; phenomena which can also be seen in figure 4.

In the case of the lead concentration increase (1000, 2000 and 3000 mg/kg Pb soil) at the same EDTA/Pb ratio = 1, the Fisher test shows a distinctly significant decrease of both biomass and maize plant height.

This aspect of biomass and plant height development in this type of treatment can be observed in Figures 3 and 4.

A distinctly significant decrease of the two parameters (biomass and height) is observed in the experimental variant of 1000 mg/kg Pb soil; this again excludes the EDTA/Pb = 1 ratio even for this experimental variant of 1000 mg/kg Pb soil.

The increase in the concentration of Pb in the leaf is significant in all variants, but the evolution of biomass is also decisive and how it decreases significantly at all three concentrations compared to the blank, the EDTA/Pb = 1 variant can not be taken into account calculation.

CONCLUSIONS

EDTA application does not influence hyperaccumulation. The toxicity of 3000 mg Pb/kg is too high and the plant does not tolerate this toxicity. Thus, another ligand/lead ratio has to be chosen and other solutions are sought to stimulate plant growth and increase the accumulation of metals in the plant.

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